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On May 12, 2006

TOWNSEND and TOWNSEND and CREW LLP

By: Patricia Andrus

PATENT
Attorney Docket No.: 016976-000610US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No. 8602
CHANG et al.	Examiner: Brian A. Whiteman
Application No.: 10/383,834	Technology Center/Art Unit: 1635
Filed: March 6, 2003	DECLARATION OF QIANG XU, PH.D. UNDER 37 C.F.R. §1.132
For: LACTOBACILLI EXPRESSING BIOLOGICALLY ACTIVE POLYPEPTIDES AND USES THEREOF	
Customer No.: 20350	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Qiang Xu, Ph.D., being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I received my Ph.D. in the field of Plant Physiology from Kansas State University in 1991. I am currently a Director, MucoCept Research at Osel, Inc., the assignee of the present patent application. I have been in this position since 2004.

3. I am a named inventor of the present patent application and I have reviewed the patent application as well as the office action dated November 15, 2005. The claims of the patent application are directed to methods of expressing a biologically active protein by contacting a mucosal surface with a *Lactobacillus jensenii* bacterium recombinantly altered to express a biologically active protein. I understand that the Examiner has rejected the claims as allegedly obvious in view of Tagliabue *et al.*, (WO 96/11277) alone, or in view of Tagliabue *et al.*, Boyd and Vallor *et al.*, or in further combination with Lee. In spite of previous arguments that those of skill in the art would find it unpredictable which species of *Lactobacillus* could be transformed, the Examiner has maintained the rejection. Specifically, the Examiner stated that "the applicant has not provided facts to rebut the presumption of operability of genetically engineering *L. jensenii* to express a biologically active protein." *See*, November 15, 2005 Office Action, page 9. This declaration has been prepared to demonstrate that as of the priority date (March 8, 2002) of the present application, it was unpredictable which transformation protocol, if any, would be effective to genetically engineer *L. jensenii* to express a biologically active protein.

4. To my knowledge, as of the priority date of the present patent application (March 8, 2002), no one had reported actual transformation of *L. jensenii*. Indeed, as discussed in detail in the amendment dated August 22, 2005, there were several reports in the scientific literature that other *Lactobacillus* species could not be successfully transformed. Therefore, one of ordinary skill in the art would not have assumed that any particular protocol was effective to transform *L. jensenii*. Instead, it is my opinion that it was unpredictable as of the priority date of the present patent

application what protocol, if any, would be effective to transform *L. jensenii*. As discussed below, it took considerable effort by the inventors of the present patent application to determine conditions that were effective in generating transformed *L. jensenii*.

5. The inventors of the present patent application made initial attempts to transform *L. jensenii* using several published transformation protocols that had been used successfully for other *Lactobacillus* species. Protocols tested included those described in Bringel *et al.*, *Plasmid* 22:193-202 (1989) and Wei *et al.*, *J. Microbiol. Methods* 21:97-109 (1995). In our experiments, neither of these protocols resulted in successful transformation of *L. jensenii*, further demonstrating that it was not a simple or predictable matter to transform *L. jensenii*.

6. An additional electroporation protocol was identified in Luchansky *et al., J. Dairy Sci.* 74:3293-3302 (1991). Luchansky *et al.* teaches transformation of *L. acidophilus* using a specific electroporation protocol involving plasmid ligation mixtures. See, Luchansky *et al.*, page 3296, paragraph spanning left and right columns. It should be noted at this point that to the extent the Tagliabue *et al.* describes *any* transformation procedure, it also involved transformation with ligation mixtures. *See*, Tagliabue *et al.*, page 11). No transformation experiment we have performed using plasmid ligation mixtures or using standard methods (Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, NY) as described in Tagliabue *et al.* have ever resulted in successful transformation of *L. jensenii*. Thus, following the exact protocol and type of DNA (plasmid ligation mixtures) described in Luchansky *et al.* or Tagliabue *et al.* does not result in effective transformation of *L. jensenii*.

7. To achieve transformation of *L. jensenii*, we used intact purified plasmids instead of the ligation mixtures as described in Luchansky *et al.* In addition,

instead of using 0.4 cm interelectrode gap cuvettes, per the Luchansky *et al.* protocol, we chose 0.2 cm cuvettes to transform *L. jensenii*. Transformation efficiency of *L. jensenii* was affected by interelectrode gap. *L. jensenii* was cultured to reach 0.7 at OD600 in MRS broth. Cells were washed in sterile distilled H2O and resuspended. Two hundred microliters of competent cells (about $7x10^8$ CFU) were electroporated in 952 mM sucrose and 3.5 mM $MgCl_2$ with 1 μg intact purified plasmid DNA, 2.5 kV, and 200 ohms. After electroporation, bacteria were plated on the erythromycin-containing MRS plates for 24 hours. Then, the erythromycin resistant colonies were counted. As shown in Table I below, this cuvette modification resulted in approximately an eight-fold increase in erythromycin resistant colonies compared to the cuvette size used by Luchansky *et al.*

Table I

Interelectrode gap cuvette (cm)	Time constant	Number of colonies
0.4	4.7	100
0.2	3.76	790
0.1	5.22	0

9. In view of the forgoing, it is clear that merely following protocols described in the prior art for transforming other *Lactobacillus* species was not effective for transforming *L. jensenii*. It is my scientific opinion that transformation of *L. jensenii* was both unpredictable and difficult prior to the significant experimentation we carried out to generate the data included in the present application. Therefore, I do not believe it was obvious for one of ordinary skill in the art how to transform *L. jensenii* as of the priority date of the present application.

Date: 5/11/2006 By: Qiang Xu

Qiang Xu, Ph.D.